REPORT OF THE COMMITTEE ON THE DESCRIPTIVE CHART FOR 1919

H. J. CONN, *Chairman*, H. A. HARDING, I. J. KLIGLER, W. D. FROST, M. J. PRUCHA, AND H. N. ATKINS

Presented at the Boston Meeting of the Society of American Bacteriologists, December 29, 1919

PART I. METHODS OF PURE CULTURE STUDY. REVISED

The 1917 report of this committee contained a description of the methods recommended to be used with the Descriptive Chart. Copies of the report have been on sale by the Society and the first edition is now practically exhausted. Opportunity is therefore taken to revise the report, printing it in the JOURNAL OF BACTERIOLOGY to call it to the attention of the members of the Society, the reprints to be placed on sale as before. As soon as these reprints are available, one copy will be furnished with every order for Descriptive Charts, and additional copies may be obtained for 15 cents apiece postpaid, or if five or more copies are ordered, for 10 cents each, transportation not postpaid. Orders for these reports, as well as for the Descriptive Charts, are to be sent to the chairman of the committee (address Geneva, N. Y.).

Methods are given in this report for all the determinations listed on the 1917 chart (recommended for instruction) but methods have not yet been prepared for all the determinations called for by the older chart. As the chart designed for instruction is now in most general use, it has seemed wisest to give further attention to these few methods rather than to do work on some of the other, less frequently used, determinations. The methods given here are not to be considered official. They are merely the best that have come to the attention of the committee at the present time. Criticisms and suggestions are at all times welcome, in order that future editions of this report may be brought up-to-date.

PREPARATION OF MEDIA

Beef-extract broth shall have the following composition:

Beef-extract	3 grams
Peptone	5 grams
Distilled water	1000 cc.

Beef-extract agar shall be of the same composition plus the addition of 12 grams of oven-dried agar or 15 grams of commercial agar. *Beef-extract gelatin* shall be of the same composition, with the addition of 120 grams of Gold Label gelatin, or 100 grams of some brand of gelatin (such as "Bacto-gelatin," or United States Glue Company gelatin) having greater jellying power. These media are to be made up according to the directions given by the committees of the American Public Health Association on water analysis and on milk analysis (1916, 1917), except that white of egg may be used for clarification if desired. It is recommended, however, that instead of using phenolphthalein in adjusting the reaction of these media the simpler and more accurate procedure be adopted of adjusting to the neutral point of brom thymol blue.[1] Bring the media to such an acidity as to turn this indicator a distinct grass-green (neither yellow green nor blue green). This color indicates approximately "true neutrality," i.e., a hydrogen-ion concentration between pH = 6.6 and pH = 7.4. Another equally satisfactory method of adjusting media to this hydrogen-ion concentration is to bring them to such an acidity as to cause the first faint trace of permanent pink to appear with phenol red.[2]

Sugar broths. Just before sterilization 1 per cent of the required carbohydrate is to be added to beef-extract broth. Otherwise proceed as for sugar-free broth. Adjust reaction with brom thymol blue or phenol red.

Plain gelatin. Proceed as for beef-extract gelatin, but omit beef-extract and peptone. Clarify with white of egg.

Nitrate broth. For routine work add 0.1 per cent KNO_3 to the above formula for beef-extract broth. Routine *nitrate agar* should contain 0.1 per cent KNO_3 added to the ordinary formula

[1] Use a 0.04 per cent solution of brom thymol blue in 95 per cent alcohol.

[2] Use a 0.02 per cent solution of phenol red in 95 per cent alcohol.

for beef-extract agar. Modification of these routine formula is often necessary, as explained below (see p. 130).

Starch agar. Add 0.2 per cent of water-soluble starch to the ordinary beef-extract agar.

Indicator media. Saccharine media with some indicator to show acid production are frequently used. Litmus is the most common indicator, enough of which should be added in saturated aqueous solution to give the medium a distinct blue color (taking care that the litmus solution used is not so alkaline as to alter, appreciably, the reaction of the medium). Litmus, however, does not give accurate results in terms of hydrogen ion concentration; so except for certain special purposes (see p. 130) it is recommended that brom cresol purple be used to detect increase in acidity, and cresol red to detect increase in alkalinity. It is convenient to keep these indicators in concentrated alcoholic solutions of such strength that 1 cc. will be sufficient for each litre of medium. For this purpose a 1.6 per cent solution of either indicator in 95 per cent alcohol is recommended. Brom cresol purple is purple in neutral or alkaline media and is yellow in acid media. Cresol red is yellow at neutrality and in acid media, turning red under alkaline conditions. Under certain circumstances it is desirable to have an indicator that will show a change in either direction from neutrality. Brom thymol blue does this, but is not satisfactory in media because its range is too short to distinguish differences between different kinds of bacteria. Very satisfactory results, however, may be obtained with a mixture of brom cresol purple and cresol red (0.5 cc. each of 1.6 per cent alcoholic solution of the two dyes to the litre of medium.) This mixture changes very slowly from purple to yellow through a long range (from about pH = 8.0 to about pH = 5.0) extending to a considerable distance on both sides of neutrality. By comparing with a blank tube of the neutral medium, it is very easy to detect an increase in either acidity or alkalinity.

Recently certain other combinations of indicators have been recommended for this same purpose. Bronfenbrenner (1919) recommends a combination of china blue with rosolic acid or preferably its sodium salt, and Morishima (1920) a combination

of china blue with phenol red. Both of these combinations are colorless or nearly so at pH = 7.0, turning blue as the reaction becomes acid and red as it becomes alkaline. Rosolic acid has the advantage over phenol red of having a more alkaline range (pH = 7.3 to pH = 9) than phenol red, hence giving the Bronfenbrenner combination a sensitive range from pH = 5 to pH = 9. Rosolic acid is insoluble in water, but it is possible to keep it in concentrated alcoholic solution (as above recommended for brom cresol purple and cresol red) so that only 1 cc. of alcohol is added to a liter of medium. The concentration of china blue in the medium should be 0.0025 per cent, that of rosolic acid or its sodium salt 0.005 per cent, while in combination with china blue a 0.001 per cent solution of phenol red is recommended. Either of these combinations should have distinct advantage over the combination of brom cresol purple with cresol red; but there has been as yet no opportunity to compare them.

Variations of these media. For certain organisms the above formulae are not the best—many pathogenic bacteria, for instance require more peptone than is provided in the above formula for broth, while some organisms are best studied in media of a hydrogen-ion concentration different from that recommended above. In such cases the individual investigator is free to modify the media to suit his own purposes; but whenever other than these routine formulae are used, the fact should be stated on the chart. In employing a reaction other than that of neutrality it is recommended that instead of using the titrimetric method, the reaction be adjusted to some definite shade of brom cresol purple, if a more acid reaction is desired, or of phenol red if it is to be more alkaline.

Optional media. In many laboratories other media than those specifically mentioned on the chart are in general use, such as potato, blood serum, agar stabs, and so forth. Blank spaces are left on the chart for recording characteristics on any optional media.

INVIGORATION OF CULTURES

Provided a medium can be found upon which the organism to be studied grows vigorously, it should always be invigorated before study, even though freshly isolated from its natural habitat. The procedure to employ is as follows:

Prepare duplicate sub-cultures in standard glucose broth, and on standard agar slopes, placing cultures of each at 37° and 25°C. On the basis of the resulting growth the organism falls into one of the following series:

Series I. Organisms which produce good growth (surface growth, distinct turbidity, or heavy precipitate) in twenty-four hours at 37° in glucose broth.

Series II. Organisms which do not produce good growth in twenty-four hours as above, but do in forty-eight hours at 25° in glucose broth.

Series III. Organisms which do not grow well in glucose broth but do produce good growth on the surface of agar in twenty-four hours at 37°.

Series IV. Organisms excluded from the above groups but which produce good growth on the surface of agar in forty-eight hours at 25°.

Record the series number on the chart at the proper place and proceed with the invigoration by inoculating into another tube of glucose broth for organisms of series I and II, or of standard agar for organisms of series III and IV. Incubate this tube at the temperature, and for the time, called for by the series in which it belongs; then transfer from this tube to a third tube and incubate as before. From this third culture make a gelatin or agar plate and incubate at the temperature previously used until colonies of sufficient size for isolation are obtained. Transfer from a typical colony to one or more agar slants and incubate for one day at 37° or for two days at 25° according to the temperature relation of the organism studied.

In case the organism does not produce vigorous growth on either of these media at either temperature, it should be invigorated with any medium and at any temperature known to be

adapted to its growth. Under such circumstances invigorate by the procedure just outlined but using the medium and temperature found most favorable for the organism in question, recording on the chart the method of invigoration adopted. If no conditions are known under which the organism in question produces vigorous growth, it should be studied without preliminary cultivation as soon as possible after isolation from its natural habitat. Such an organism is not likely to give good growth on any ordinary media, and the results of the study called for by the chart will have little significance.

STUDY OF MORPHOLOGY

The routine study of morphology should be from dried preparations, stained with fuchsin, methylen blue, or gentian violet. Preparations to show the vegetative cells should be made, preferably, from agar slant cultures, from a few hours to two days old, according to the rapidity of growth. The medium and temperature used and the age of the culture should be recorded.

Motility. Hanging-drop preparations of young broth or agar cultures should be examined for motility. If motile, microscopic preparations should be made to show the arrangement of the flagella, using any of the ordinary methods of flagella staining with which the student can obtain good success. Even if motility is not observed in hanging-drop, it is wise to attempt a flagella stain, because motile organisms often lose their motility under the conditions of observation. Even negative results from both hanging-drop preparation and flagella stain do not absolutely prove that the organism is immotile.

Presence of spores. Routine examinations for spores should be made on stained, dried preparations from agar slant cultures a week old. Stain with methylen blue. Vegetative forms take the stain, but spores do not. In most cases there will be no trouble in finding spores if the organism produces them. All rather large rods however, (0.8 micron or more in diameter) should be regarded as possible spore-producers, even though microscopic examination does not show spores. Such bacteria should be

mixed with sterile water and heated to 85°C. for ten minutes; if still alive, spores may be regarded as unquestionably present. Also make repeated transfers of the culture onto agar and examine at various ages. A culture of a large rod should not be recorded as a non-spore-former unless all these tests are negative.

Capsules. An organism should not be recorded as having capsules unless they have been actually stained by one of the methods of capsule-staining described in bacteriological text books.

Irregular forms. Forms that differ from the typical shape for the organism (i.e., "involution forms," etc.) such as branching forms, clubs, spindles or filaments should be noted and sketched.

Special stains. Of these the Gram stain has been given particular attention by the committee and at present the Stirling modification is recommended. Further work is being done at present upon an improved method of making this stain, which will be discussed in the next number of the JOURNAL. The Stirling modification is carried out as follows:

Prepare gentian violet solution by grinding 5 grams in 10 cc. of 95 per cent alcohol in a mortar. Add 2 cc. anilin oil, distilled water 88 cc. Filter.

Iodine solution is as usual: 1 gram iodine, 2 grams potassium iodide, 300 cc. water.

The procedure recommended in the 1918 report was as follows: one minute in stain; wash in water; one minute in iodine solution; wash in water; two minutes in absolute alcohol; wash in water; thirty seconds in counter stain (10 cc. saturated alcoholic safranin in 90 cc. water). This procedure gives very good results; but recent work undertaken in the army cantonments shows that equally good results can be obtained by the following rapid method: one to five seconds in stain; wash in water; five to ten seconds in iodine solution; wash in water; ten to twenty seconds in absolute alcohol; wash in water; five to ten seconds in counterstain.

We are informed that the Stirling solution of gentian violet can be made more stable by mixing normal hydrochloric acid with the anilin oil before dissolving in water. (Add 0.5 cc. normal HCl to 2 cc. anilin oil; dissolve in 88 cc. water and filter;

mix with 10 cc. of alcoholic gentian violet prepared as above.) The committee has not yet tested out this procedure.

Sketches. Drawings of all the morphological characteristics should be made on the blank spaces on the chart to the right of the descriptions. Both typical and atypical forms should be sketched, using care to designate which are typical.

CULTURAL CHARACTERISTICS

Cultures for the study of cultural characteristics should be incubated at 37°C. in case of organisms of series I and III, and at 25° in case of organisms of series II and IV, except that gelatin cultures should be incubated at 20°. Room temperature may be used in place of 25° at certain seasons of the year; but if a minimum thermometer shows that the temperature falls below 22° during the course of the work, note should be made of the fact. On the day when good growth first appears the proper descriptive terms on the card should be underlined; after subsequent study, the changes should be noted in the space provided, and sketches of the different stages should be made.

PHYSIOLOGY

Liquefaction of gelatin. Old method. The method in most common use is to hold gelatin stab cultures six weeks at 20°C. Plain gelatin should be used.

Provisional method. It is recommended that the following method proposed by Rothberg (1917) be put in provisional use until experience shows its value. It is designed to distinguish "true liquefiers" (organisms producing ecto-enzymes) from the organisms that produce endo-enzymes of proteolytic action that are released from the cell after death and cause liquefaction of the gelatin if incubated for the long period mentioned above. The method is to give the organism a preliminary cultivation for eighteen to forty-eight hours (according to its rapidity of growth) in a 1 per cent solution of gelatin at 25° or 37° according to its temperature relations; then inoculate on surface of gelatin in test tube and incubate fifteen days at 20°.

Relation to free oxygen. Provisional method. Determine by noting the presence or absence of growth in open and closed arm, respectively, of fermentation tubes containing glucose broth. Care must be taken to use fermentation tubes from which the dissolved oxygen has been recently driven off by heating. In case of gas production, this test is of comparatively little value, because bubbles of gas may carry the sediment up with them; hence if an organism produces gas from glucose, the test should, if possible, be made in the presence of some other sugar which it attacks (acidifies) without gas-formation. It must be remembered, however, that even anaerobes do not grow in the absence of free oxygen except in the presence of a chemical substance (such as carbohydrate) which they are able to reduce and use as a source of oxygen.

Fermentation of sugars and glycerol. This is normally to be studied in fermentation tubes. Ordinarily use beef-extract broth containing 1 per cent of the substance investigated; but if the organism does not grow well in such broth and some medium is known in which it does grow well, the latter may be used. Generally speaking, organisms of series I and II should be studied in broth, organisms of series III and IV in some other medium. Incubate organisms of series I and III at 37°, organisms of series II and IV at 25°. Test ordinarily on 1st, 3rd, and 7th days, although the best days for testing will depend upon the rapidity of growth of the culture. Hence on the chart, although space is given for recording reaction on three separate days, blanks are left for the individual student to fill in with the days upon which the tests are actually made. Inoculations should always be made at least in triplicate.

To test for acid, it is recommended that in place of the illogical titrimetric method, determinations of hydrogen-ion concentration be made by the colorimetric method described by Clark and Lubs (1917 a). In accurate research work the exact shade of the indicator should be compared with that obtained in standard "buffer" solutions, and results recorded in terms of pH. In laboratories where these standard solutions cannot be obtained, it is better to record results simply as + or −, according to the

reaction of the culture to litmus, than to use the titration method. Under such conditions it is possible, however, to obtain a rough idea of the hydrogen-ion concentration by the use of Clark and

TABLE 1

Degrees of acidity easily recognized in clear media

ACIDITY	INDICATOR REACTIONS	APPROXIMATE pH-VALUE
"Neutral"	Blue or green to brom thymol blue*	Over 6.2
"Weak"	Yellow to brom thymol blue Purple to brom cresol purple*	5.2–6.0
"Moderate"	Yellow to brom cresol purple Orange to methyl red†	4.6–5.0
"Strong"	Maximum red to methyl red Blue or green to brom phenol blue*	3.2–4.4
"Very strong"	Yellow to brom phenol blue	Under 3.0

* Use a 0.04 per cent alcoholic solution.
† Use a 0.02 per cent alcoholic solution.

TABLE 2

Degrees of acidity easily recognized in milk

ACIDITY	INDICATOR REACTION, ETC.	APPROXIMATE pH-VALUE
"Neutral"	Same color with brom cresol purple* as sterile milk; i.e., blue to gray green	6.2–6.8
"Weak"	Color with brom cresol purple lighter than in sterile milk; i.e., gray-green to greenish yellow	5.2–6.0
"Moderate"	Yellow with brom cresol purple. Not curdled	4.7–6.0
"Strong"	Curdled. Blue or green to brom phenol blue*	3.2–4.6
"Very strong"	Yellow to brom phenol blue	Under 3.0

* Use a 0.04 per cent alcoholic solution.

Lubs' series of indicators without making accurate determinations of pH. Four different degrees of acidity can be easily distinguished by this simple method in sugar broth with an initial reaction of neutrality. The indicator reactions for these differ-

ent degrees of acidity are listed in table 1, together with the approximate range of pH to which each corresponds. In the absence of accurate determinations, these degrees of acidity may be recorded by the indefinite terms, "weak," "moderate," "strong and "very strong," or by the symbols +, ++, +++, and ++++. If the student desires to record increase in alkalinity in the same table on the chart, he can use the symbol 0 for neutrality and − for an alkaline reaction.

Gas production is ordinarily measured in percentage of gas in the closed arm, and the ratio of H: CO_2 by means of absorption with NaOH, using the methods described in laboratory manuals (filling open arm with 4 per cent NaOH, allowing gas to enter open arm, shaking and returning gas to closed arm). As this method is far from accurate, it is recommended for provisional use only.

The fermentation test is ordinarily of no significance for organisms of series III and IV because of their poor growth in broth. Sometimes these organisms can be studied in some other liquid medium in which they do give good growth; but generally it is preferable to use agar slants. In such a case, use a sugar agar containing brom cresol purple, china blue, or a mixture of indicators as suggested on page 129. Increase in acidity can be detected by fading of the purple color of the brom cresol purple or by the appearance of blue if china blue is used. Gas-production can usually be detected in agar cultures by the presence of cracks and air bubbles, but as a test for gas, agar slants are not as reliable as fermentation tubes.

A more detailed discussion of hydrogen-ion concentration and of methods for determining acid-production is given in the 1918 report of this committee, copies of which can be obtained from the chairman.

Milk. Acid production in milk can be detected by adding brom cresol purple to the culture and comparing with the color obtained by adding the same proportionate quantity of indicator to sterile milk. (Brom thymol blue does not give satisfactory results in milk.) Four degrees of acidity that can be simply recognized in milk are listed in table 2. They correspond closely

to those listed in table 1, differing only in that brom cresol purple is used instead of brom thymol blue to show "neutrality" and that the curdling point (pH = 4.7) is used to separate between "moderate" and "strong" acidity instead of the less definite point of maximum red to methyl red. The same methods of expression used in recording acidity in clear media should be used in recording that of milk.

Litmus milk often gives valuable information, showing not only the production of acid, but also decolorization of the litmus by organisms that are able to reduce it. More accurate results as to acidity can be obtained by using brom cresol purple, as shown by Clark and Lubs (1917 b). This indicator, however, does not show the reduction phenomena which are sometimes of diagnostic value in litmus milk cultures; its substitution for litmus is not, therefore, always to be recommended.

Reduction of nitrates. The following procedure is recommended: Inoculate first into nitrate broth and onto slants of nitrate agar, the media having the composition given on p. 128. Test the cultures on various days as indicated on the chart. On these days examine first for gas as shown by foam in the broth or by cracks in the agar. Then test for nitrite with the following reagents:

(1) Dissolve 8 grams sulphanilic acid in 1 litre of 5 N acetic acid (1 part glacial acetic acid to 2.5 parts of water), or in 1 litre of dilute sulphuric acid (1 part concentrated acid to 20 parts water).

(2) Dissolve 5 grams α-naphthylamine in 1 litre of 5 N acetic acid or of very dilute sulphuric acid (1 part concentrated acid to 125 parts water).

Put a few drops of each of these reagents in each broth culture to be tested, and on the surface of each agar slant. A distinct pink or red in the broth or agar indicates the presence of nitrite. It is well to test a sterile check which has been kept under the same conditions, to guard against errors due to absorption of nitrite from the air. Presence of nitrite or of gas shows the nitrate to have been reduced. A negative result does not prove that the organism is unable to reduce nitrates; in such a case further study is necessary, as follows:

In case the fault seems to lie in poor growth, search should be made for a nitrate medium in which the organism in question does make good growth by means of the following modifications: increasing or decreasing the amount of peptone; altering the reaction; adding some readily available carbohydrate. Presence of nitrite or gas in any nitrate medium whatever should be recorded as nitrate-reduction. Unless the routine formula is used, the exact composition of the medium must always be given.

If the organism grows well and yet produces no nitrite or gas, the determination must be recorded as doubtful unless the organism can grow well in some synthetic medium containing no nitrogen except nitrate. It is recommended that such an organism be tested in a medium containing small quantities of phosphate, calcium, chlorine, etc., with KNO_3 as a source of nitrogen and sucrose as a source of energy and of carbon.[3] Such a medium generally allows good growth with an organism capable of utilizing nitrate and sucrose. Unfortunately neither glucose nor lactose can be used in this medium as a source of carbon and energy, for the ordinary "c.p." preparations of these sugars contain much ammonia. If the organism in question grows (even but slightly) on a synthetic medium of this sort, it should be tested for nitrite by the usual method and for ammonia by means of Nessler's reagent (comparing with an uninoculated tube as a check). The presence of nitrite, of ammonia (i.e., a more pronounced ammonia reaction than in check tube), or of gas indicates nitrate-reduction.

The production of gas (free N) from nitrate is not a very common one; but a considerable number of soil organisms have this power, and one should be on the lookout for it in studying soil bacteria. The agar slant test is ordinarily a sufficiently delicate test; but, if liquid media are used, more reliable results may be obtained by the use of fermentation tubes.

Chromogenesis. Color production should be recorded if observed in broth, on beef-extract agar, gelatin or potato, or if

[3] An illustration of such a medium which has proved satisfactory for some bacteria is: K_2HPO_4, 0.5 gram, $CaCl_2$, 0.5 gram, KNO_3, 1 gram, sucrose 10 grams, agar 12 grams, water 1000 cc.

noticed to a striking extent on any other medium. In the group number, the point devoted to chromogenesis refers to the color produced on beef-extract agar.

Diastatic action on starch. Provisional method. Use beef-extract agar containing 0.2 per cent of soluble starch. Pour into a petri dish, and after hardening make a streak inoculation on its surface. Incubate at 37° for organisms of series I and III, at 25° for organisms of series II and IV. Determinations for the group number shall be based upon results obtained on the seventh day. To make the test, flood the surface of the petri dishes with a saturated solution of iodine in 50 per cent alcohol. The breadth of the clear zone outside of the area of growth indicates the extent of diastatic action. If over 2 mm. in width on the seventh day it shall be recorded as "strong;" if under 2 mm. as "feeble;" if no clear zone is present, as "absent."

This method requires some modification, e.g., reducing the amount of peptone in the medium, for organisms that grow so rapidly as to cover the entire surface of the plate in seven days, thus leaving no room for a clear zone outside.

The group-number is a brief means of recording the salient features of the organism. It is primarily a summary of the physiological characteristics just discussed. As each of the determinations is made, the proper figure for that place in the group number is to be checked or underscored. After completing the determinations, the entire group number is to be written in at the place left for it on the chart. The genus symbol should precede the group number. The present group number, adopted by the Society in 1907, was intended for use with the generic names of Migula. As Migula's genera are not in such general use today as they were ten years ago, a revision of the group number on some other basis is now being undertaken by the committee, and will be discussed in Part II of this report.

Brief characterization. On the right hand margin of page one of the chart is a place for recording by a + or − sign other important characteristics of the organism (primarily cultural)

not included in the group number. This margin together with the group number constitute a brief characterization of the organism—a summary of the tests outlined above.

REFERENCES

American Public Health Association. 1916 Standard methods for the examination of water and sewage.

American Public Health Association 1917 Standard methods of bacteriological analysis of milk. Am. J. Pub. Health, **6**, 1315–1325.

BRONFENBRENNER, J. J. 1919 J. Med. Research, **39**, p. 25.

CLARK, W. M. AND LUBS, H. A. 1917 a The colorimetric determination of hydrogen-ion concentration. J. Bact. **2**, 1–34, 109–136, 191–236.

CLARK, W. M. AND LUBS, H. A. 1917 b A substitute for litmus for use in milk cultures. J. Agric. Research, **10**, 105–111.

MORISHIMA, K. 1920 Phenol red china blue as an indicator in fermentation tests of bacterial cultures. J. Inf. Dis. **26**, 43–44.

ROTHBERG, W. 1917 Observations on some methods for the study of gelatine liquefaction. Read before Soc. Amer. Bacteriologists, December, 1917. See Abs. Bact. **2**, 12.

GLOSSARY OF TERMS USED ON THE CHART

Adherent, Applied to sporangium wall, indicates that remnants of sporangium remain attached to endospore for some time.

Aerobic, growing in the presence of free oxygen; strictly **aerobic,** growing *only* in the presence of free oxygen.

Amorphous, without visible differentiation in structure.

Anaerobic, growing in the absence of free oxygen; **strictly anaerobic,** growing *only* in the presence of free oxygen; **facultative anaerobic,** growing both in presence and in absence of free oxygen.

Arborescent, branched, tree-like growth.

Beaded, (in stab or stroke culture) disjointed or semi-confluent colonies along the line of inoculation.

Bipolar, at both poles or ends of the bacterial cell.

Brittle, growth dry, friable under the platinum needle.

Butyrous, growth of butter-like consistency.

Chains, four or more bacterial cells attached end to end.

Chromogenesis, the production of color.

Ciliate, having fine, hair-like extensions, resembling cilia, sometimes not visible to the naked eye.

Clavate, club-shaped.

Coagulation, the separation of casein from whey in milk.

Contoured, an irregular, smoothly undulating surface, like that of a relief map.

Convex, surface the segment of a sphere.

Crateriform, a saucer-shaped liquefaction of the medium.

Cuneate, wedge-shaped.

Curled, composed of parallel chains in wavy strands, as in anthrax colonies.
Diastatic action, conversion of starch into simpler carbohydrates, such as dextrins or sugars, by means of diastase.
Echinulate, a growth along line of inoculation with toothed or pointed margins.
Effuse, growth thin, veily, unusually spreading.
Endospores, thick-walled spores formed within the bacterial cell; i.e., typical bacterial spores like those of *B. anthracis* or *B. subtilis.*
Entire, with an even margin.
Erose, border irregularly toothed.
Filaments, applied to morphology of bacteria, refers to thread-like forms, generally unsegmented; if segmented, to be distinguished from chains (q.v.) by the absence of constrictions between the segments.
Filamentous, growth composed of long, irregularly placed or interwoven threads.
Filiform, in stroke or stab cultures, a uniform growth along line of inoculation.
Flocculent, containing small adherent masses of bacteria of various shapes floating in the culture fluid.
Fluorescent, having one color by transmitted light and another by reflected light.
Granular, composed of small granules.
Infundibuliform, form of a funnel or inverted cone.
Iridescent, exhibiting changing rainbow colors in reflected light.
Lobate, having the margin deeply undulate, producing lobes (see *undulate*).
Luminous, glowing in the dark, phosphorescent.
Maximum temperature, temperature above which growth does not take place.
Membranous, growth thin, coherent, like a membrane.
Minimum temperature, temperature below which growth does not take place.
Mycelioid, colonies having the radiately filamentous appearance of mold colonies.
Napiform, liquefaction in form of a turnip.
Opalescent, resembling the color of an opal.
Optimum temperature, temperature at which growth is most rapid.
Papillate, growth beset with small nipple-like processes.
Pellicle, bacterial growth forming either a continuous or an interrupted sheet over the culture fluid.
Peptonization, rendering curdled milk soluble by the action of peptonizing enzymes.
Peritrichiate, covered with flagella over the entire surface.
Persistent, lasting many weeks or months.
Plumose, a fleecy or feathery growth.
Polar, at the end or pole of the bacterial cell.
Pulvinate, decidedly convex, in the form of a cushion.
Punctiform, very small, but visible to naked eye; under 1 mm. in diameter.
Radiate, showing ray-structure.
Raised, growth thick, with abrupt or terraced edges.
Reduction, removing oxygen from a chemical compound. Refers to the conversion of nitrate to nitrite, ammonia, or free nitrogen, and to the decolorization of litmus.
Rhizoid, growth of an irregular branched or root-like character, as in *B. mycoides.*
Ring, growth at the upper margin of a liquid culture, adhering to the glass.
Rapid, developing in twenty-four to forty-eight hours.

Rugose, wrinkled.
Saccate, liquefaction in form of an elongated sac, tubular, cylindrical.
Slow, requiring five or six days for development.
Spindled, larger at the middle than at the ends. Applied to sporangia, refers to the forms frequently called **clostridia.**
Sporangia, cells containing endospores.
Spreading, growth extending much beyond the line of inoculation, i.e., several millimeters or more.
Stratiform, liquefying to the walls of the tube at the top and then proceeding downwards horizontally.
Transient, lasting a few days.
Truncate, ends abrupt, square.
Turbid, cloudy with flocculent particles; i.e., cloudy plus flocculence.
Umbonate, having a button-like, raised center.
Undulate, border wavy, with shallow sinuses.
Viscid, growth follows the needle when touched and withdrawn; sediment on shaking rises as a coherent swirl.